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# The diverse roles of mononuclear phagocytes in prion disease pathogenesis

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**Keywords:** mononuclear phagocytes, dendritic cells, macrophages, PrP<sup>Sc</sup>, TSE, prion transmission

**Abbreviations:** BSE, bovine spongiform encephalopathy; CNS, central nervous system; DC, dendritic cell; FDC, follicular dendritic cell; GALT, gut-associated lymphoid tissue; LC, Langerhans cell; LN, lymph node; LRS, lympho-reticular system; MNP, mononuclear phagocyte; PrP, prion protein; TSE, transmissible spongiform encephalopathy

Transmissible spongiform encephalopathies (TSEs) or prion diseases, are neurological diseases that can be transmitted through a number of different routes. A wide range of mammalian species are affected by the disease. After peripheral exposure, some TSE agents accumulate in lymphoid tissues at an early stage of disease prior to spreading to the nerves and the brain. Much research has focused on identifying the cells and molecules involved in the transmission of TSE agents from the site of exposure to the brain and several crucial cell types have been associated with this process. The identification of the key cells that influence the different stages of disease transmission might identify targets for therapeutic intervention. This review highlights the involvement of mononuclear phagocytes in TSE disease. Current data suggest these cells may exhibit a diverse range of roles in TSE disease from the transport or destruction of TSE agents in lymphoid tissues, to mediators or protectors of neuropathology in the brain.

## Transmissible Spongiform Encephalopathies (TSEs)

The TSEs are a group of fatal neurodegenerative diseases that affect both humans and animals. These diseases can occur under several different forms: spontaneous, genetic or acquired through various routes of exposure. Examples of the main disease forms and the species they affect are listed in Table 1. TSE disease is characteristically associated with vacuolation in the brain (spongiform pathology), neuronal loss, glial cell activation and amyloid deposits of the disease-associated form of prion protein (PrP), which eventually lead to neurodegeneration and death.

Despite being a neurodegenerative disease, natural transmission often occurs in the periphery before spreading to the central nervous system (CNS). Indeed, in some experimental and natural TSEs, such as natural sheep scrapie, disease is characterized by early agent accumulation in the peripheral lymphoid system.<sup>1,2</sup> However, it is worth noting that even within a single species such as the sheep, the strain of the TSE agent or *PRNP*

genotype (which encodes the cellular prion protein PrP<sup>C</sup>) of the animal can significantly influence disease pathogenesis.<sup>3,4</sup> Other forms of the disease, such as bovine spongiform encephalopathy (BSE), appear not to be associated with early TSE agent replication in the peripheral lymphoid tissue of cattle,<sup>5</sup> but PrP<sup>Sc</sup> and/or infectivity have been detected in the gut of experimentally and naturally infected cattle.<sup>6-10</sup> This difference between TSE agent strain targeting in host tissues is interesting since when BSE transmitted to other species, agent accumulation in the lymphoid tissues is a key feature of variant Creutzfeldt-Jakob disease in humans.<sup>11,12</sup>

The replication of TSE agents by host cells is critically dependent upon the expression of the host encoded, cellular isoform of the prion protein, PrP<sup>C</sup>.<sup>13</sup> PrP<sup>C</sup> is an endogenous protein which is expressed on a large range of different cell types throughout the body.<sup>14-19</sup> The normal cellular form of PrP has a mainly  $\alpha$ -helical structure<sup>20</sup> and is protease sensitive. PrP<sup>C</sup> is a 30–35 kDa glycoprotein which is anchored to the outer layer of the cell membrane through a glycosylphosphatidylinositol (GPI) anchor.<sup>21</sup> Although early studies determined that PrP<sup>C</sup> deficient mice do not appear to be adversely affected by the absence of the protein,<sup>22</sup> are not wholly immunodeficient,<sup>23</sup> and are developmentally normal,<sup>24</sup> recent studies using PrP-deficient mice show, that PrP<sup>C</sup> is associated with suppression of cognitive function caused by brain-derived amyloid- $\beta$  in Alzheimer disease,<sup>25</sup> as well as blocking pain receptors.<sup>26</sup> The protein's protective function is also associated with epilepsy,<sup>27,28</sup> and Martins et al. present the view that as well as playing a role in “loss-of-function components” in prion diseases, PrP<sup>C</sup> might also be a component in the pathogenesis of other neurodegenerative diseases.<sup>28</sup> In addition there has been some speculation about its function in the immune system, for example in mediating pro-survival signals in cells in certain circumstances such as rapid memory cell expansion,<sup>23</sup> and a potential role in T lymphocyte activation.<sup>15,29,30</sup>

TSE diseases are characteristically associated with the accumulation of insoluble aggregates of the disease-specific abnormal form of PrP within the CNS and, in some cases, within the lymphoreticular system (LRS). This abnormal form of the protein is a relatively protease resistant, predominantly  $\beta$ -pleated sheet isoform, termed PrP<sup>Sc</sup>.<sup>20</sup> Both PrP<sup>C</sup> and PrP<sup>Sc</sup> are associated with three different isoforms that are glycosylated at zero,

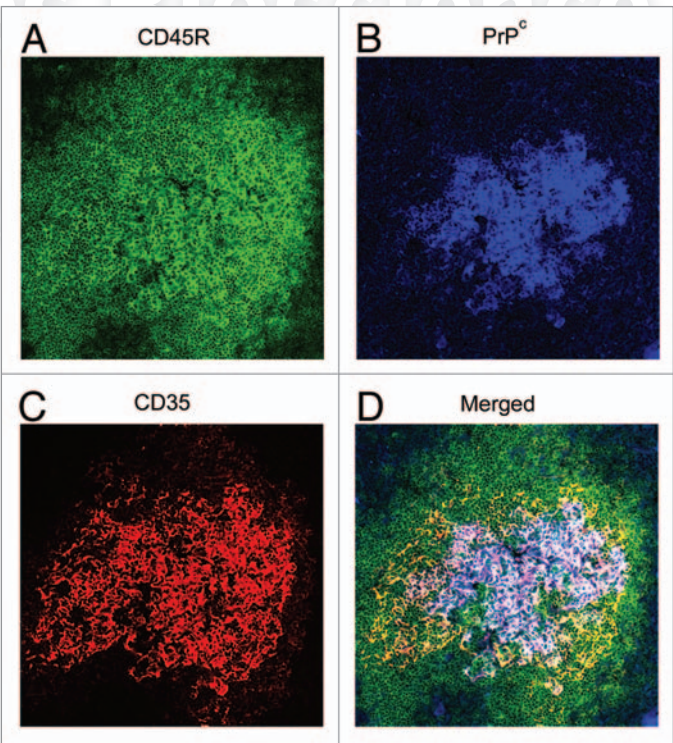
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**Table 1.** TSE diseases

TSE disease	Affected species	Route of transmission
Iatrogenic Creutzfeldt-Jacob disease	Human	Accidental medical exposure to CJD-contaminated tissues or tissue products
Sporadic Creutzfeldt-Jacob disease (sCJD)	Human	Unknown. Somatic mutation to spontaneous conversion of PrP <sup>c</sup> to PrP <sup>Sc</sup> ?
Variant Creutzfeldt-Jacob disease (vCJD)	Human	Ingestion of BSE-contaminated food or blood transfusion from CJD-infected blood donor
Familial Creutzfeldt-Jacob disease	Human	Germline mutations of the <i>PRNP</i> gene
Gerstmann-Straussler-Scheinker syndrome	Human	Germline mutations of the <i>PRNP</i> gene
Kuru	Human	Ritualistic cannibalism
Fatal familial insomnia	Human	Germline mutations of the <i>PRNP</i> gene
Bovine Spongiform encephalopathy	Cattle	Ingestion of contaminated feed
Scrapie	Sheep, goats	Acquired, ingestion, horizontal transmission, vertical transmission unclear
Chronic wasting disease	Elk, deer, moose	Acquired, ingestion, horizontal transmission, vertical transmission unclear
Transmissible mink encephalopathy	Mink	Acquired (ingestion) source unknown
Feline spongiform encephalopathy	Domestic and zoological cats	Ingestion of BSE-contaminated food
Exotic ungulate encephalopathy	Nyala, Kudu	Ingestion of BSE-contaminated food

one or both of the protein's two possible glycosylation sites, situated at residues 181 and 197 of human PrP, and residues 180 and 196 in murine PrP.<sup>31</sup> According to the “prion hypothesis,”<sup>32</sup> PrP<sup>Sc</sup> is the infectious agent in TSE disease and it is this protein that causes PrP<sup>c</sup> to convert to the disease associated isoform. Quaking-induced conversion (QUIC),<sup>33</sup> and protein misfolding cyclic amplification (PMCA),<sup>34</sup> are methods which allow for the amplification of protein, in a similar manner to DNA amplification by polymerase chain reaction (PCR).<sup>35</sup> These techniques have enabled propagation of infectious PrP using infected brain-derived or recombinant PrP. As well as studying PrP conversion, these techniques have enabled the detection of infectious TSE agents in various tissues at dilutions previously undetected by other methods. This had only been performed in the presence of additional cofactors such as nucleic acids and lipids, but recently, infectious prions were generated by PMCA from recombinant hamster prion protein without any apparent cofactors.<sup>36</sup> Data derived from the use of these two techniques lend further weight to the argument of a protein hypothesis in disease transmission.<sup>36</sup>

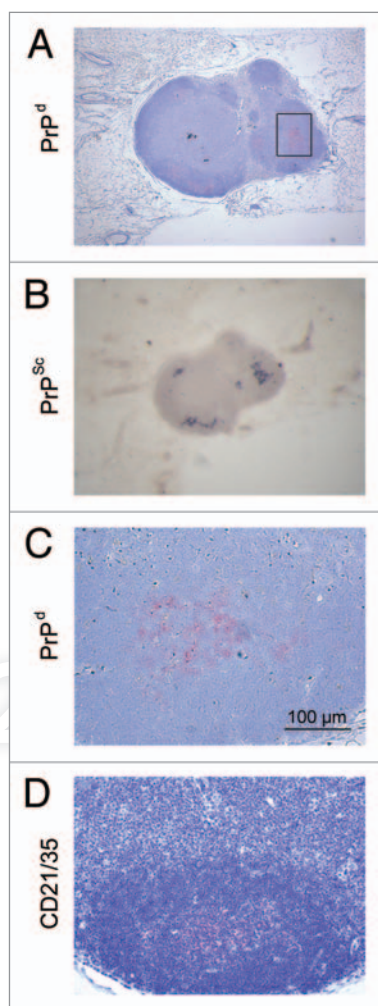
An important question that still surrounds these diseases is how the TSE agent is transported from the site of infection to the LRS and onwards into the CNS. Much speculation has surrounded the issue of whether the TSE agent is transported via cell-associated or cell-free mechanisms. Studies in certain species show that when LRS involvement occurs during the early stages of TSE infection, PrP<sup>Sc</sup> accumulates first on PrP<sup>c</sup>-expressing follicular dendritic cells (FDCs).<sup>4,37-41</sup> FDCs are stromal-derived, tissue resident cells, found in the germinal centers of lymphoid follicles.<sup>42</sup> These cells express high levels of PrP<sup>c</sup> on their surface (Fig. 1), and are clearly associated with PrP<sup>Sc</sup> accumulation in peripheral lymphoid tissues (Fig. 2). The FDC has since been identified as an important site of TSE agent accumulation and replication in the LRS.<sup>19,43-45</sup> Furthermore, in the absence of FDCs, TSE agent neuroinvasion



**Figure 1.** PrP<sup>c</sup> is strongly expressed on FDCs in the lymphoid follicles of the spleen. Images taken from mouse spleen immunolabelled with the anti-CD45R [green, (A)], anti-PrP (1B3) [blue, (B)] and anti-CD35 [red, (C)] antibodies. FDCs and CD35-expressing B cells detected with the anti-CD35 specific antibody. B cells detected with the anti-CD45R specific antibody and PrP<sup>c</sup> expression was detected using the 1B3 polyclonal antibody.<sup>126</sup> (D) merged image of all three antibodies.

is impaired and disease susceptibility reduced. FDCs are considered to amplify TSE agents above the threshold required for neuroinvasion.





**Figure 2.** PrP<sup>Sc</sup> accumulates in the draining LN following scrapie infection via the skin. (A) PrP<sup>d</sup> was detected with the anti-PrP specific antibody 6H4 in the draining inguinal LN five weeks post scrapie infection. (B) Paraffin embedded tissue (PET) blot analysis of adjacent sections confirms PrP<sup>d</sup> accumulations to be proteinase K resistant PrP<sup>Sc</sup>. (C) Enlarged image of PrP<sup>d</sup> labeling from boxed area of (A). (D) Shows location of FDCs via immunostaining with the anti-CD21/CD35 specific antibody. PrP<sup>Sc</sup> is accumulating on FDCs in the LNs.

### Mononuclear Phagocytes (MNP)s and TSE Pathogenesis

Even though peripheral lymphoid tissues, and the FDCs within them, have been identified as important sites of TSE agent replication prior to neuroinvasion, little is known of how TSE agents are transported to these sites or what, if any, cells are involved. MNPs are a diverse group of hematopoietically-derived phagocytic cells which includes classical dendritic cells (DCs) and macrophages, but also Langerhans cells (LCs) in the epidermis of the skin and microglia in the brain. The distribution of these cells at the body's surfaces (intestine, skin, mucosa, etc.,) and their ability to phagocytose antigens and deliver them to draining lymphoid tissues, suggested MNPs may either destroy TSE agents or transport them within the host.

The precise ontogeny of classical DCs and macrophages is the subject of much debate. Indeed, there is growing evidence that these two cell types are not as distinct from each other as was once thought.<sup>46-48</sup> These cells are often identified or isolated based solely on the expression of a small set of cell surface markers, such as CD11c/Itgax for DCs,<sup>49</sup> and CD207/langerin for LCs.<sup>50-53</sup> Even under the traditional “umbrella terms” of DCs and macrophages more distinct cellular subsets have been described based on the expression of a long list of different markers. However, the expression of these and other markers has been shown to be less specific than was originally considered.<sup>46,54</sup> For example, the temporary depletion of CD11c<sup>+</sup> cells in CD11c-DTR transgenic mice, originally considered to specifically deplete DCs,<sup>55</sup> has been shown to also deplete many other macrophage subsets, such as the CD169<sup>+</sup> macrophages in the spleen and lymph nodes (LN) (Fig. 3).<sup>46,54</sup> Much of the data described in this review was generated from in vitro experiments utilizing bone marrow-derived DCs. However, the recent meta-analysis of a large collection of gene expression data from a range of mouse leukocyte lineages shows bone marrow-DCs were clearly identified as phagocytes (macrophages) and were transcriptionally distinct from tissue classical DCs. Indeed, there are few mRNA markers that clearly distinguish classical DCs from macrophages other than low expression of those required for phagocytosis.<sup>48</sup> Clearly, without also testing their biological properties (phagocytosis, ability to stimulate naïve T cells, etc.) it is difficult to accurately distinguish between classical DCs and macrophages based solely on the expression of a limited set of surface markers. This is especially true in immunohistochemistry-based studies. While the authors considered it important to highlight the ambiguity surrounding the discrimination of individual MNP populations, we stick to the traditional terms of macrophages or DCs within this review to avoid further confusion!

### Classical DCs: Taxis for TSE Agents?

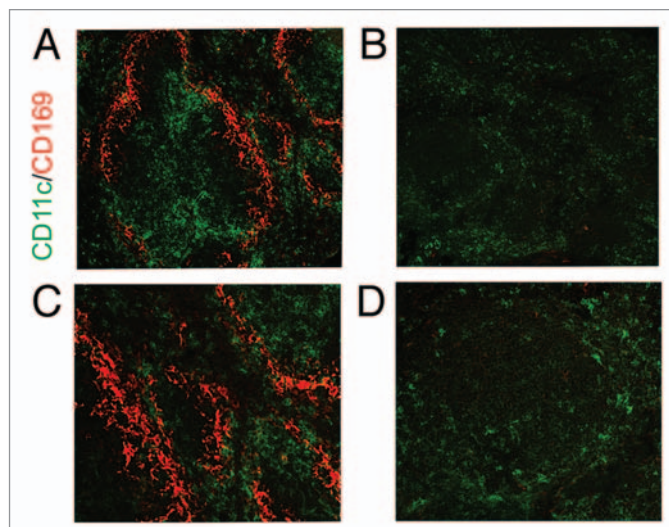
Haematopoietic, classical DCs are a distinct cell type from mesenchymal-derived FDCs.<sup>42</sup> Classical DCs are antigen presenting cells (APCs) and as such they sample their natural environment for foreign antigens, which they process and transport to the nearest lymphoid tissues to initiate a specific immune response. Classical DCs take up antigen through endocytosis or sometimes macropinocytosis.<sup>56</sup> Classical DCs travel via the lymphatic system to the secondary lymphoid organs in response to chemokine stimulation where they present antigen to lymphocytes.<sup>57</sup> Although the primary role of DCs is to present antigens to T cells, they are also capable of presenting them to B cells. This sometimes occurs in the form of intact antigen, in contrast to the processed antigen presented to T cells.<sup>58</sup> These characteristics therefore identify classical DCs as possible candidates for the transport of the intact TSE agents from the site of exposure to the peripheral lymphoid system. Indeed, studies show the retention of PrP<sup>Sc</sup> within bone marrow derived DCs 72 h after in vitro exposure to PrP<sup>Sc</sup>-enriched scrapie associated fibrils.<sup>59</sup> However, another study has also suggested that bone marrow-derived DCs rapidly degrade PrP<sup>Sc</sup>,<sup>60,61</sup> but as we described above, these cells

are highly phagocytic when compared with classical DC from tissues.

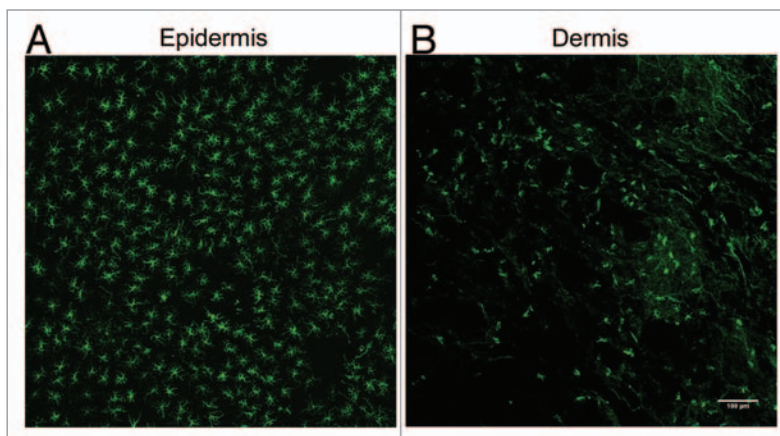
Classical DCs could influence TSE pathogenesis at a variety of stages in the disease process: transport from the site of infection to the LRS in the case of LRS involvement; transport between cells within the lymphoid tissues; or the transfer between the lymphoid tissues and the nervous system (neuroinvasion). The argument for a DC-related role in TSE pathogenesis is strengthened by the evidence that the prion protein fragment 106–126 functions as a chemoattractant to immature monocyte-derived DCs.<sup>62</sup> Evidence for a possible role of DCs in the delivery of TSE agents to lymphoid tissues was provided by using a transgenic mouse model in which CD11c<sup>+</sup> cells<sup>63</sup> or CD8<sup>+</sup>CD11c<sup>+</sup> cells<sup>64</sup> were significantly reduced. The absence of these cells at the time of intraperitoneal scrapie infection significantly prolonged the incubation period of the disease when compared with wild type controls.<sup>63,64</sup>

### DCs in the Gut

The oral route is considered a major route of exposure for many naturally acquired TSEs such as natural sheep scrapie, BSE, chronic wasting disease in cervids and variant CJD (vCJD) in man. Studies of experimental transmissions in mice and sheep naturally affected with scrapie show TSE agents accumulate first in the gut-associated lymphoid tissue (GALT) such as the Peyer's patches and mesenteric LN.<sup>4</sup> The accumulation of TSE agents upon FDCs within the GALT is crucial for the efficient spread of disease to the brain.<sup>37,40</sup> Studies have also investigated the role of DCs in the transport of the TSE agent from the intestinal lumen to the GALT.<sup>59,65</sup> Huang et al.<sup>59</sup> used thoracic duct cannulation to collect the cells and lymph draining the intestines of rats intra-intestinally injected with PrP<sup>Sc</sup>. Data from this study showed that intestinal DCs could acquire and transport PrP<sup>Sc</sup> from the intestinal lumen, to the mesenteric LN, via the lymph, within hours of intestinal exposure. Furthermore, using CD11c-DTR transgenic mice in which CD11c<sup>+</sup> cells can be specifically depleted,<sup>55</sup> it was shown that an absence of CD11c<sup>+</sup> cells at the time of oral infection blocked the early TSE agent accumulation in the GALT and spleen. Additionally, the overall susceptibility to scrapie disease was reduced in mice where the CD11c<sup>+</sup> cells were depleted prior to scrapie challenge. These data imply that CD11c<sup>+</sup> cells play a crucial role in the transport of the scrapie agent from the intestinal lumen to the GALT. Whether these data indicate a role for classical DCs or macrophages remains to be determined, since recent analysis of the CD11c-DTR mouse shows that all the MNPs within the gut lamina propria are depleted in this model.<sup>46</sup> The migration of MNPs, to and within lymphoid tissues, is regulated by the expression of various chemokines. However, the dissemination of TSE agents from the intestine to the GALT is not dependent on cell migration through the chemokine/receptor system CCL19/CCL21/CCR7.<sup>66</sup>



**Figure 3.** Depletion of DCs and macrophages in the spleen of the CD11c-DTR mouse. (B and D) CD11c<sup>+</sup> DCs are partially depleted in this transgenic mouse line through the injection of Diphtheria toxin. CD169<sup>+</sup> macrophages in the spleen are also depleted through their low level expression of CD11c. (A and C) Normal expression of CD11c and CD169 in control mice. (C and D) are higher magnification images of (A and B).



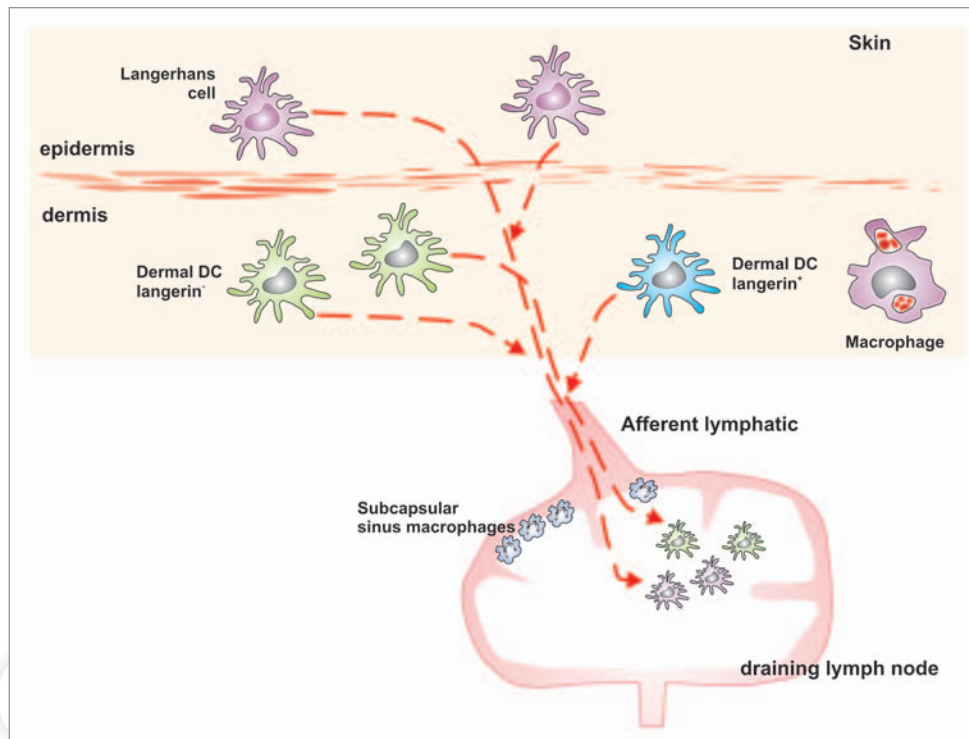
**Figure 4.** Detection of LCs and langerin<sup>+</sup> dermal DCs in the mouse ear following immunofluorescent labeling of epidermal and dermal sheets. (A) LCs form a dense network of cells within the epidermis. (B) Langerin<sup>+</sup> dermal DCs are not abundant within the dermis as the LCs in the epidermis. A small number of these cells may also be migrating LCs.

### Skin and Mucosa

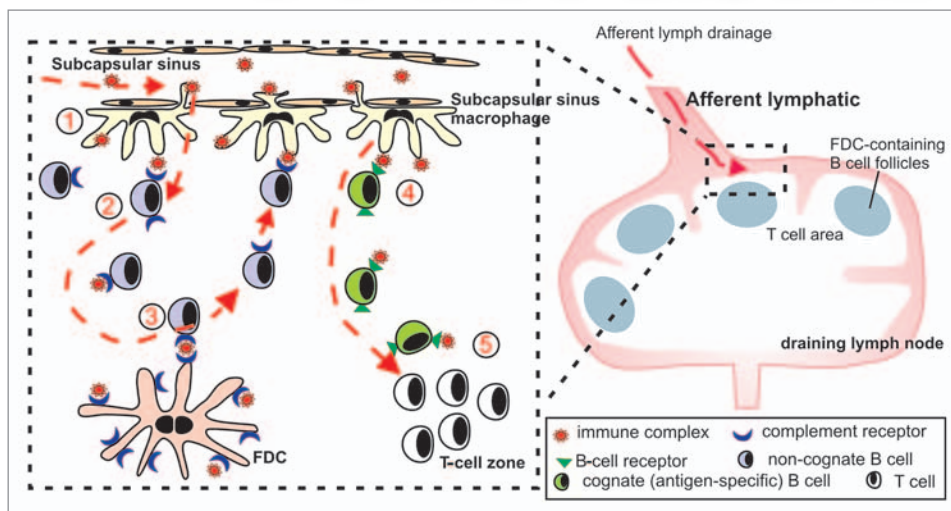
TSE agents can be readily transmitted via lesions to the skin<sup>67-70</sup> and oral mucosa.<sup>71-73</sup> Thus, natural TSE infections might also occur via lesions in the mouth and gastrointestinal tract through consumption of rough feed or birth-associated lesions to the skin or mucus membranes. Furthermore, TSE agent infectivity has also been identified in the skin,<sup>74-76</sup> as well as in antler velvet.<sup>77</sup>

The skin provides a first line of defense against infection and is therefore the primary site of residence for a number of MNPs that help to maintain this barrier. These MNPs include LCs in the epidermis and classical dendritic cells and macrophages in





**Figure 5.** Schematic representation of the MNPs in the skin of the mouse. LCs can be found in the epidermis. The dermis is situated below the epidermis and comprises langerin<sup>+</sup> as well as langerin<sup>-</sup> DCs and various macrophage populations. All these cell types migrate from the skin to the draining LN, and therefore may play a potential role in the transport of the TSE agent from the skin to the draining LN, where PrP<sup>Sc</sup> accumulates following infection via the skin.



**Figure 6.** Schematic representation of the role of subcapsular sinus macrophages in the transport of immune complexes to FDC in the LN. (1) Subcapsular sinus macrophages capture lymph borne immune complexes in the subcapsular sinus which they transcytose intact across their surfaces to underlying follicular B cells. (2) Non-cognate B cells acquire the immune complexes via their complement receptors and (3) deliver them to FDCs. (4) Cognate (antigen-specific) B cells, in contrast, acquire antigen-containing immune complexes via their B-cell receptors, become activated and (5) migrate to the boundary of the T-cell zone.

the dermis (Figs. 4 and 5). Classical DCs have been suggested to transport viruses such as HIV<sup>78</sup> or Dengue<sup>79</sup> from the skin,

transmission from the skin as suggested after oral exposure. Any research looking into the role of skin DCs or macrophages in

which raises the question whether skin-derived MNPs also play a role in the uptake and transport of the TSE agent from the skin to the LRS.

Following scrapie infection via skin scarification in the mouse, agent infectivity and PrP<sup>Sc</sup> accumulation occurs first in the skin draining LN soon after exposure.<sup>67,69,80</sup> Experimental scrapie transmission in sheep via the skin failed to find a conclusive link between DCs and the transport of PrP<sup>Sc</sup>.<sup>81</sup> However, neutrophils were found to associate with PrP<sup>Sc</sup>. The definite involvement of skin resident MNPs in TSE agent transmission from the skin remains to be determined. Early research in mice also implied the lack of LC involvement.<sup>69,82</sup> However, the use of transgenic mouse models that allow for the temporary depletion of LCs, DCs or macrophages<sup>55,83</sup> prior to scrapie infection will help determine whether these cells play a role in TSE

TSE transmission needs to be put into context with the LC paradigm. Substantial heterogeneity has been revealed between MNP subtypes in different inbred mouse strains.<sup>84</sup> For example, LC density in the epidermis is much lower in C57BL/6 mice when compared with BALB/c, 129/Sv and CBA mice. Such differences could significantly contribute to the differing results observed in many host species exposed to TSE agents.

In 2007, an important novel cell type was identified in the mouse dermis: the langerin<sup>+</sup> dermal DC (Fig. 4).<sup>85-87</sup> All dermal DCs were thought to be negative for langerin expression, a marker previously considered to be exclusive to LCs. These langerin<sup>+</sup> dermal DCs have a much higher turnover rate than LCs and repopulate the skin several weeks faster than the epidermal LCs following depletion.<sup>83,88,89</sup> Upon activation, for example by skin immunization, these langerin<sup>+</sup> dermal DCs migrate from the skin to the draining LNs much earlier than the LCs.<sup>83</sup> Thus, earlier studies addressing the role of LCs would not have been able to factor in the presence of this novel cell type which may have often skewed the interpretation of data in previous studies of LC immunobiology.

Since the identification of langerin<sup>+</sup> dermal DCs, a number of publications have questioned the role of LCs in skin immunity, in particular the suggestion that they are the only APCs of the skin. Indeed, langerin<sup>+</sup> dermal DCs have been shown to be fully capable of cross-presenting antigens regardless of whether LCs are present or not.<sup>90</sup> Experiments where LC migration from the epidermis was blocked, in CD40L<sup>-/-</sup> mice or via caspase-1 inhibition, were previously used to determine whether LCs played a role in the transport of the scrapie agent from the skin to the draining LNs.<sup>69</sup> While the early accumulation of TSE agent infectivity was not affected in the draining LNs, the incubation period of disease was significantly shortened in the CD40L<sup>-/-</sup> mice. These data implied that instead of aiding pathogenesis by transporting TSE agents from the skin, LCs may impede pathogenesis by phagocytosing and degrading them.<sup>69,82</sup> In light of recent findings, further experiments are necessary to distinguish the influence of LCs and langerin<sup>+</sup> dermal DC in this process.

### Classical DCs as Mediators of Neuroinvasion

As well as being involved in the transport of the TSE agent from the periphery to the LRS, classical DCs may have a role in the act of neuroinvasion within the lymphoid tissues, for example, the transport of TSE agents to peripheral nerves. Studies show evidence of the intercellular transport of PrP<sup>Sc</sup> through tunnelling nanotubules from DCs to cultured neuronal cells.<sup>91</sup> In some TSE diseases where there appears to be little or no LRS involvement (such as BSE in cattle, or sheep of certain *PRNP* genotypes infected with scrapie) neuroinvasion occurs by an unidentified process. Whether classical DCs fulfil this role as implied in the Gousset study<sup>91</sup> is uncertain. However, studies performed in scrapie infected, FDC-deficient, TNFR1<sup>-/-</sup> mice, determined that MNPs were unlikely to have directly infected peripheral nerves within lymphoid tissues, as these mice failed to develop disease when injected with scrapie-infected CD11c<sup>+</sup> cells (classical DCs).<sup>92</sup> These results were in contrast to previous

studies in FDC-deficient RAG<sup>-/-</sup> mice, where scrapie disease was successfully transmitted.<sup>93</sup> The discrepancies between these two studies may possibly be due to much higher levels of innervation in the RAG<sup>-/-</sup> mouse spleens when compared with those from TNFR1<sup>-/-</sup> mice.<sup>92</sup>

Following replication within lymphoid tissues, TSE agents are considered to spread to the brain via peripheral sympathetic nerves.<sup>94</sup> However, it is plausible that due to their migratory nature, classical DCs carry TSE agents into the CNS across the blood-brain-barrier. Whether the immigration of TSE agent-contaminated classical DCs or circulatory monocytes into the CNS plays an important role in neuroinvasion is uncertain, as significant monocytic infiltration into the brain during TSE disease has not been reported. In sheep infected with scrapie, PrP<sup>d</sup> accumulation within specific structures in the brain, the circum-ventricular organs, was an early and consistent feature.<sup>95</sup> These sites have fenestrated capillaries, and as such are sites of molecular exchange between the CNS and the blood-stream. However, the lack of monocytic cells within these organs throughout TSE disease is consistent with the hypothesis that cell-associated haematogenous spread is not a major route of neuroinvasion.

### Macrophages: Saints or Sinners?

**Macrophages.** Macrophages typically phagocytose and degrade protein antigens more rapidly than classical DCs,<sup>96,97</sup> which can retain some protein antigens in their native form up to 36 h after exposure.<sup>98</sup> Data from in vitro studies likewise suggest that macrophages also phagocytose and degrade TSE agents including scrapie and BSE.<sup>99-101</sup> Furthermore, the depletion of macrophages before scrapie infection increased the accumulation of the scrapie agent in the spleen and accelerated disease pathogenesis.<sup>102</sup> Within the macrophage, PrP<sup>Sc</sup> was also found to colocalize with lysosomal and proteasomal proteins,<sup>103</sup> implying that, consistent with their biological characteristics, macrophages might play a preventative role in TSE disease when compared with classical DCs. However, data also imply that macrophages might transport orally acquired TSE agents within the GALT.<sup>104</sup> Whether the cells described are macrophages or classical DCs is uncertain. As highlighted earlier, it is difficult to classify these cells by immunohistochemistry based on the expression of cell surface markers alone.

**Tingible body macrophages.** Tingible body macrophages are found in close association with FDCs within the germinal centers of follicles within lymphoid tissues, where they clear proteins and apoptotic lymphocytes (tingible bodies).<sup>105</sup> Data from ultrastructural studies show high levels of PrP<sup>Sc</sup> within tingible body macrophages in lymphoid tissues of scrapie-affected mice and sheep.<sup>106-108</sup> Deposition of PrP<sup>d</sup> within tingible body macrophages in lymphoid tissues of patients with vCJD have also been described in reference 109. Recent data suggests tingible body macrophages scavenge and degrade PrP<sup>Sc</sup> following synthesis on other infected cells such as FDCs.<sup>45</sup>

**Microglia.** Microglia are the macrophages of the CNS. Gliosis, involving astrocytes and microglia, is one of the neuropathological characteristics of terminal TSE disease. Microglia

are the main source of the inflammatory response that is associated with TSE disease, and microglial activation is directly linked to the patterns of PrP<sup>Sc</sup> deposition in the brain, and precedes neuronal cell death.<sup>110,111</sup> Significant vacuolar degeneration has been detected in the microglia/macrophages of two vCJD patients.<sup>112</sup> Microglia and astrocytes have been associated with granular PrP<sup>d</sup> deposits, leading speculation that these cells play a role in processing, degrading or removing PrP<sup>Sc</sup>.<sup>113</sup> The in vitro exposure of microglia to murine scrapie brain homogenate or PrP<sup>(106–126)</sup> severely affected their phagocytic activity.<sup>114</sup> In contrast, microglia from ME7-scrapie affected mice were capable of phagocytosis, but were not able to clear PrP<sup>Sc</sup>.<sup>115</sup> Within TSE-affected brains the pro-inflammatory activity of microglia is specifically modulated by the anti-inflammatory cytokine TGFβ1. This cytokine appears to play a critical role in the downregulation of pro-inflammatory microglial responses minimizing brain inflammation and thus avoiding exacerbation of brain damage.<sup>116</sup> Whether systemic infections and inflammation lead to the dysregulation of this control and the exacerbation of neurodegeneration remains to be determined.<sup>117</sup>

A recently identified brain DC population, morphologically similar to microglia is considered to be a new member of the heterogeneous microglia population.<sup>118</sup> However, it is uncertain whether these cells are a unique subset of classical DCs. These data indicate that, as in the other tissues discussed in this review, there are a number of different MNP populations that could influence TSE pathogenesis in the brain, depending on the circumstances. Furthermore, some data might relate to a particular subset of cells, rather than to the entirety of MNPs within the brain.

**Subcapsular sinus macrophages.** Subcapsular sinus macrophages are a distinct, poorly endocytic and degradative macrophage subset.<sup>119</sup> These unique cells capture antigen-containing immune complexes arriving in the LN via their cell processes that they extend into the lumen of the subcapsular sinus.<sup>119–123</sup> In contrast to other macrophage subsets, subcapsular sinus macrophages retain immune complexes on their surfaces for rapid translocation through the floor of the subcapsular sinus to underlying, non-cognate (non-specific) follicular B cells.<sup>119</sup> These B cells then acquire the immune complexes via their complement receptors and deliver them to FDCs. The higher immune complex-binding affinities of FDCs most likely relieve the B cells of their cargo. Thus, the subcapsular sinus macrophage-B cell immune complex relay represents an efficient route through which antigens are delivered to FDCs<sup>119–123</sup> (Fig. 6). The demonstration that

subcapsular sinus macrophages in LNs (and their counterparts in the spleen) play a key role in the delivery of complement-bound immune complexes to FDCs raises the possibility that these cells might also play an important role in the transport of complement-opsonized TSE agents to FDCs within lymphoid tissues. Indeed, disease-specific PrP has been detected within the subcapsular sinus macrophages of intestinally-exposed sheep.<sup>124</sup>

## Concluding Remarks

This review aimed to highlight the potential involvement of the various MNP populations in TSE pathogenesis. Current data suggest MNPs may exhibit a diverse range of roles in TSE disease from the transport or destruction of TSE agents in lymphoid tissues, to mediators or protectors of neuropathology in the brain. Much of the research described above studies the influence of MNPs on TSE pathogenesis during steady-state conditions. However, under inflammatory conditions MNPs may exacerbate TSE pathogenesis, for example through the release of neurotoxic mediators in the brain<sup>117</sup> or the transmission of disease between individuals through the contamination of bodily secretions such as milk.<sup>125</sup> While some MNPs may play important roles in TSE pathogenesis, it is equally likely that in some circumstances their involvement is minimal. The route of TSE infection, strain of TSE agent and host species may all influence the role of MNPs in disease pathogenesis. Indeed, it is equally probable that in some instances TSE agents reach the draining lymphoid tissues via a cell-free mechanism such as in a complement-bound immune complex or via the conduit system.<sup>123</sup> Recent advances in immunology have identified a long list of new members of the MNP phagocyte system, which may also have an important impact on TSE pathogenesis. Continuing research with the new tools and models available will help to unravel the mysteries that still surround TSE pathogenesis.

## Disclosure of Potential Conflicts of Interest

The authors declare no financial conflict of interest.

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